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Alterations in Composition of Field- and Laboratory-Developed Estuarine Benthic Communities Exposed to Di-n-Butyl Phthalate

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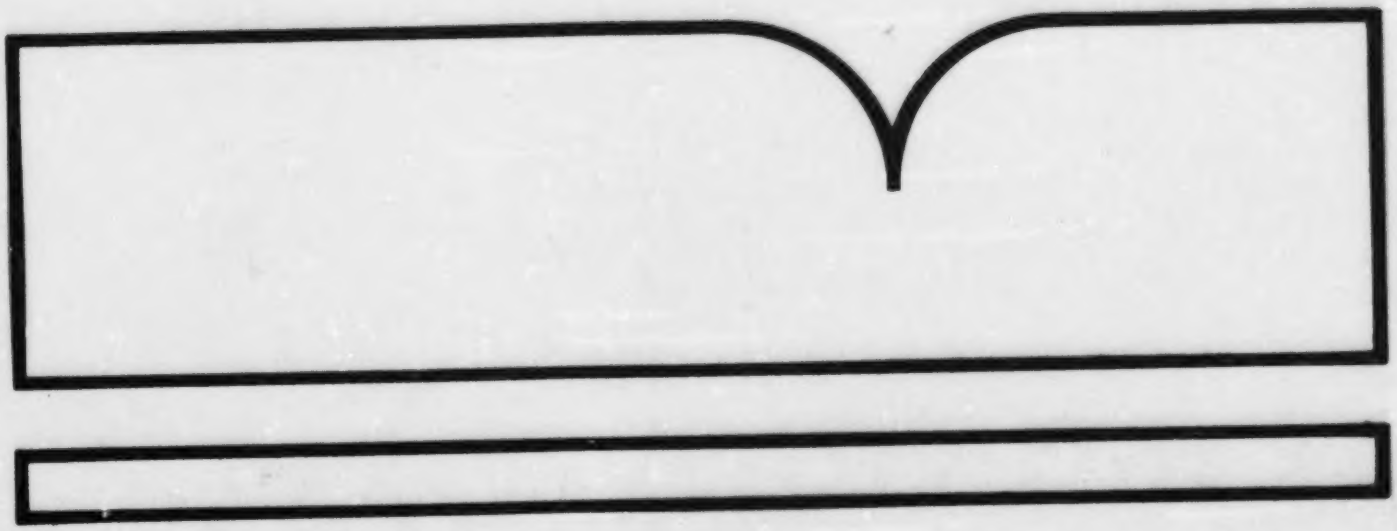

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16. ABSTRACT

Macrobenthic animal communities, developed in sand-filled aquaria in the laboratory and in the field, were exposed to three concentrations of the plasticizer, di-n-butyl phthalate (DBP), and effects on community structure assessed. Laboratory communities were colonized by planktonic larvae in unfiltered seawater; field communities, by naturally occurring animals. After 8 weeks of colonization, laboratory and field communities (removed to the laboratory) were exposed to DBP for 2 weeks. The numbers of individuals and species of animals in aquaria receiving 3.7 mg DBP/ℓ (laboratory-colonized) or 3.8 mg DBP/ℓ (field-colonized) were significantly less than those in control aquaria or in aquaria that received lower concentrations of the plasticizer. Affected phyla in laboratory or field faunal assemblages were chordates, mollusks, arthropods, and annelids. Amphipods, Corophium acherusicum, collected only from laboratory communities, also were significantly fewer in communities exposed to 0.34 mg DBP/ℓ. Density of individuals and numbers of species were not affected by 0.04 mg DBP/ℓ.

Key words: Phthalates, macrobenthos, benthic communities, aquatic pollution, toxicity tests.

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ALTERATIONS IN COMPOSITION OF FIELD- AND LABORATORY-DEVELOPED ESTUARINE BENTHIC COMMUNITIES EXPOSED TO DI-*n*-BUTYL PHTHALATE*

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Macrobenthic animal communities developed in sand-filled aquaria in the laboratory and in the field were exposed to three concentrations of the plasticizer, di-*n*-butyl phthalate (DBP), and effects on community structure were assessed. Laboratory communities were colonized by planktonic larvae in unfiltered sea water; field communities, by naturally occurring animals. After 8 wk of colonization, laboratory and field communities (removed to the laboratory) were exposed to DBP for 2 wk. The numbers of individuals and species of animals in aquaria receiving 3.7 mg DBP/l (laboratory-colonized) or 3.8 mg DBP/l (field-colonized) were significantly less than those in control aquaria or in aquaria that received lower concentrations of the plasticizer. Affected phyla in laboratory or field faunal assemblages were chordates, mollusks, arthropods, and annelids. Amphipods, *Corophium acherusicum*, collected only from laboratory communities, also were significantly fewer in communities exposed to 0.34 mg DBP/l. Density of individuals and numbers of species were not affected by 0.04 mg DBP/l.

Key words: phthalates; macrobenthos; benthic communities; aquatic pollution; toxicity tests

INTRODUCTION

Phthalate esters are a large class of synthetic organic chemicals (esters of ortho benzene dicarboxyl acid) widely used as plasticizers (added to plastic resins such as polyvinyl chloride). Along with a high rate of production, about 5×10^8 kg annually in the United States, significant amounts of phthalates are entering the marine environment (Giam et al., 1978). They are common in industrial and municipal wastes. Surface water collected in the Gulf of Mexico off the Mississippi River contained 0.6 mg/l di-2-ethylexyl phthalate (DEHP); lesser amounts also occurred in waters off Escambia Bay near Pensacola, Florida (Corcoran, 1973).

Although phthalates have a relatively low acute toxicity to aquatic organisms, prolonged exposure to low concentrations may be detrimental. Mayer and Sanders (1973) found that waterfleas (*Daphnia magna*) continuously exposed to 0.003 mg

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DEHP/l for a complete life cycle of 21 days exhibited significantly reduced reproduction, acute values (LC_{50}) for aquatic invertebrates were 700 to 3300 times higher than the chronic value.

Di-*n*-butyl phthalate (DBP) is one of the more abundantly produced and widely used phthalate plasticizers, and is also used as an insect repellant. Ninety-six hour LC_{50} values, in mg/l, for freshwater animals are 2.1 for an amphipod (*Gammarus pseudolimnaeus*), >10 for a crayfish (*Orconectes nais*), and range from 0.73 to 6.47 for four species of fish (Mayer and Sanders, 1973). DBP is also accumulated by aquatic organisms. For example, it was accumulated by *G. pseudolimnaeus* during a 14-day exposure at a level 6700 greater than the 0.0001 mg/l concentration in water (Sanders et al., 1973). The only work on responses of saltwater animals to DBP known to us is that of Laughlin et al. (1977), who found that 1 mg/l had no detectable effect on the entire larval development of the mud crab, *Rhithropanopeus harrisii*. To obtain information on the effects of DBP on the estuarine environment, we conducted studies on the responses of laboratory- and field-colonized benthic communities exposed to this chemical.

MATERIALS AND METHODS

Experimental communities

The effects of DBP on macrobenthic animals that colonized sand-filled aquaria for 8 wk (October 5 to November 30, 1981) were determined by comparing community structures in aquaria treated with DBP for 2 wk (December 1 to 15, 1981) with those in untreated aquaria. Laboratory aquaria were colonized by settling of planktonic larvae entrained in continuously-supplied unfiltered sea water from Santa Rosa Sound, Florida, while field aquaria in Santa Rosa Sound were colonized by naturally occurring animals. Field aquaria were located in 3 m of water, about 10 m from the pump-intake that supplied sea water to the laboratory aquaria. Salinity and temperature of sea water entering the laboratory, recorded continuously, averaged 29.5‰ (25.0 to 33.5‰) and 20°C (16 to 26°C) during colonization.

Eight aquaria each were used for the control and for three exposure concentrations of DBP (nominal, 0.05, 0.5 and 5 mg/l) for the laboratory and the field-colonized communities. The eight aquaria for each treatment consisted of four $32 \times 32 \times 6$ cm deep boxes divided diagonally in half and grouped by four in the form of a square (Fig. 1). Aquaria were constructed of acrylic plastic using plastic dissolved in ethylene dichloride as the adhesive. They were filled with clean silica sand (particle size, 0.2 to 0.8 mm) dredged previously from Santa Rosa Sound. In the laboratory, groups of four boxes were placed in each of four box frames (Fig. 1) that, when filled, contained a 3-cm layer of water over the sand. Unfiltered sea water was delivered continuously to a splitter box where four adjacent glass tubes supplied water at 1.5 l/min to the center of each frame. Water flowed from the

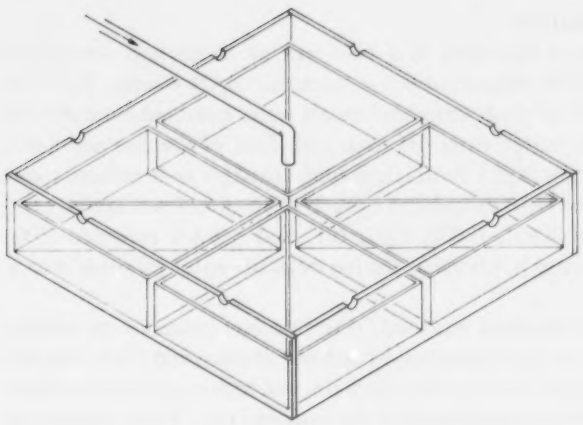

Fig. 1. Arrangement of 8 triangular aquaria used per treatment to test the effects of DBP on estuarine macrobenthic communities. Sea water was continuously supplied at 1.5 l/min to the center of each frame; phthalate was introduced through a side opening (not shown) in the tubing that carried sea water.

frame through notched openings on all sides. Photoperiod was 12 L : 12 D; light was supplied by four 1.2-m fluorescent lamps (34 W). Concurrent with the laboratory study, SCUBA divers placed 16 boxes in four groups of four in the field. The boxes, positioned in the substratum so that their surfaces were level with the surrounding sand, were covered with 6.4-mm (0.25 inch) hardware cloth to exclude large predators, such as crabs.

After colonization in the field for 8 wk, the boxes were collected by divers and covered with acrylic plastic for immediate transfer to the laboratory. In the laboratory, field boxes were placed in box frames that received sea water as described above; each group of four divided boxes (eight aquaria) consisted of one box randomly selected from each of the four field groups.

DBP (100% pure), dissolved in a stock solution consisting of 60% acetone and 40% distilled water, was metered by pump through flexible tubing and discharged through a side opening in the glass tubing that carried sea water to the center of each box frame that received DBP. An equal amount of carrier solvent (0.5 ml/l) was metered into the water that entered the control frames. Water samples, taken thrice weekly from each frame during the 2-wk exposure, were analyzed for DBP content. A peristaltic tubing pump was used to obtain 1-liter samples simultaneously from the eight frames over a period of 1 h. Data are presented as average measured concentrations. Salinity during exposure averaged 30‰ (27 to 32‰); temperature, 15.0°C (11.5 to 19.0°C).

After 2 wk of exposure to DBP, the communities were harvested. Animals retained by a 1-mm mesh sieve were preserved and identified.

Chemical and statistical analyses

To extract DBP, 1 liter of sea water at a flow rate of 10 ml/min was passed through a 6×0.5 cm i.d. glass column of 0.5 g Amberlite XAD-4 resin. The resin was then washed with 10 ml of fresh water and excess water removed from the column by vacuum. DBP was eluted from the resin with 5 ml of acetone and quantitated by electron-capture gas-liquid chromatography. Tandem resin columns were used when concentrations of DBP exceeded 0.1 mg/l. Recoveries from samples fortified at concentrations of 0.001, 0.005, 0.05, 0.1, and 5 mg/l exceeded 95%. Solubility of DBP in sea water is 4.0 mg/l; the log octanol–water partition coefficient (log *P*), 4.0.

The SAS (1979) statistical package was used (1) to calculate means of the number of individuals and species for each treatment by site and phylum and (2) to perform overall comparisons among the control, low, medium, and high exposure concentrations using the Kruskal–Wallis nonparametric (or ranking) test. When statistically significant treatment effects were detected, a nonparametric multiple comparison procedure was used to determine which pairs of concentration exposures were different (Hollander and Wolfe, 1973). In all tests, the level of significance was $\alpha = 0.05$.

RESULTS

Laboratory-colonized communities

The averages and SD of DBP concentrations measured in water from laboratory-developed communities were nondetectable (control), 0.044 mg/l ± 0.014, 0.34 mg/l ± 0.12, and 3.7 mg/l ± 0.70.

A total of 2331 animals representing 29 species of 6 phyla were collected from laboratory-colonized aquaria (Table I). The chordate, *Molgula manhattensis*, was most abundant; other dominant animals were *Laevicardium mortoni* (mollusk), *Corophium acherusicum* (arthropod), *Armandia agilis* (annelid), and *Leptosynapta inhaerens* (echinoderm).

Community structure was significantly altered by 3.7 mg DBP/l (Table II). Average numbers of individuals and species of chordates, mollusks, arthropods, and annelids per aquarium were less in the highest concentration than those in the control and in the lowest concentration. Only 11 individuals and 4 species were collected from aquaria exposed to 3.7 mg/l, compared to 715 to 833 animals and 19 to 25 species in control aquaria and aquaria that received lower concentrations of the toxicant. No *M. manhattensis*, the dominant species harvested in this study (2 to 30 mm in diameter), survived the highest concentration. Deaths among mollusks and annelids began on the second day of exposure to 3.7 mg/l. Observed deaths on the sixth day of exposure included 15 Morton's egg cockles (*L. mortoni*, 4 to 11 mm high), 3 yellow cockles (*Trachycardium muricatum*, 5 to 7 mm high), 2 opisthobranchs (*Bursatella leachii*, 8 to 13 mm long), and 33 polychaete worms (*A.*

TABLE I

Animals in laboratory-colonized communities collected from control aquaria and from aquaria that received DBP for 14 days. Replicates were pooled.

Taxon	Control	DBP (mg/l)			Total animals
		0.044	0.34	3.7	
Chordata					
Molgula manhattensis	393	454	462	0	1309
Mollusca					
Laevicardium mortoni	190	191	160	0	541
Acteocina canaliculata	12	17	18	3	50
Musculus lateralis	6	7	5	2	20
Diastoma varium	7	4	3	0	14
Trachycardium muricatum	2	6	2	0	10
Bursatella leachii pleii	4	1	1	0	6
Anadara transversa	0	2	2	0	4
Polycera hummi	1	1	2	0	4
Mitrella lunata	0	1	1	0	2
Lyonsia hyalina	1	0	0	0	1
Tellina sp.	0	1	0	0	1
Total mollusks	223	231	194	5	653
Arthropoda					
Corophium acherusicum	94	75	8	0	177
Annelida					
Armandia agilis	28	32	25	1	86
Sthenelais articulata	4	2	1	0	7
Capitella capitata	3	3	0	0	6
Amphictene sp.	1	2	1	0	4
Mediomastus californiensis	1	1	0	0	2
Owenia fusiformis	0	1	1	0	2
Prionospio heterobranchia	1	0	1	0	2
Arenicola cristata	0	1	0	0	1
Malacoceros vanderhosti	0	1	0	0	1
Neanthes succinea	0	1	0	0	1
Polydora sp.	0	1	0	0	1
Unidentified Flabelligeridae	0	0	1	0	1
Total annelids	38	45	30	1	114
Echinodermata					
Leptosynapta inhaerens	21	24	19	5	69
Unidentified Holothuroidea	1	3	2	0	6
Ophiophragmus filograneus	1	0	0	0	1
Total echinoderms	23	27	21	5	76
Coelenterata					
Unidentified Actiniaria	1	1	0	0	2
All phyla					
Individuals	772	883	715	11	2331
Species	20	25	19	4	29

agilis, 9 to 17 mm long). Heights of 66 dead Morton's egg cockles measured after the 2-wk exposure to 3.7 mg/l averaged 5 mm (2 to 11 mm).

The population of the amphipod, *C. acherusicum*, the only species of arthropod, also was significantly decreased by 0.34 mg DBP/l. Only 8 individuals occurred, compared to 94 in the control.

Field-colonized communities

The averages and SD of DBP concentrations measured in water from field-developed communities were nondetectable (control), 0.036 mg/l ± 0.005, 0.45 mg/l ± 0.14, and 3.8 mg/l ± 0.62.

A total of 181 animals representing 46 species of 7 phyla were collected from field-colonized aquaria (Table III). Mollusks and annelids dominated in number; relatively few animals from other phyla were collected.

Significantly fewer individuals and species occurred in 3.8 mg/l than in lower concentrations and the control (Table IV). The number of mollusks, but not annelids, was significantly reduced by the highest concentration. Only three species of mollusks survived in aquaria that received 3.8 mg DBP/l, compared to 11 species in control aquaria.

TABLE II

Average density of animals and average number of species (in parentheses) per aquarium collected from laboratory-colonized control aquaria and from aquaria that received DBP for 14 days.

Phylum	Control	DBP (mg/l)		
		0.044	0.34	3.7
Chordata	43.5	56.8	57.8	0*
	(1.0)	(1.0)	(1.0)	(0*)
Mollusca	27.9	28.9	24.3	0.6*
	(3.6)	(3.8)	(3.4)	(0.6*)
Arthropoda	11.8	9.4	1.0*	0*
	(1.0)	(1.0)	(0.5)	(0*)
Annelida	4.8	5.6	3.8	0.1*
	(2.1)	(2.6)	(1.6)	(0.1*)
Echinodermata	2.9	3.4	2.6	0.6
	(1.0)	(1.3)	(1.1)	(0.4)
Coelenterata	0.1	0.1	0	0*
	(0.1)	(0.1)	(0)	(0)
All phyla	96.5	104.1	89.4	1.4*
	(8.9)	(9.8)	(7.6)	(1.1*)

* Significantly different from control at the 5% level.

TABLE III

Animals in field-colonized communities collected from control aquaria and from aquaria that received DBP for 14 days. Replicates were pooled.

Taxon	Control	DBP (mg/l)			Total animals
		0.036	0.45	3.8	
Mollusca					
Diastoma varium	3	5	6	0	14
Laevicardium mortoni	6	3	5	0	14
Tellina sp.	4	6	2	0	12
Anomalocardia auberiana	4	2	4	0	10
Marginella apicina	2	2	1	1	6
Morula didyma	1	1	4	0	6
Anadara transversa	1	1	1	0	3
Mitrella lunata	1	0	2	0	3
Crassostrea virginica	0	0	2	0	2
Eupleura sulcidentata	1	0	1	0	2
Mangelia quadrata	1	0	1	0	2
Thais haematoma	0	2	0	0	2
Bursatella leachii pleii	0	0	0	1	1
Atrina rigida	0	0	0	1	1
Polinices duplicatus	1	0	0	0	1
Total mollusks	25	22	29	3	79
Annelida					
Haploscoloplos robustus	4	1	2	4	11
Tharyx marioni	4	1	0	3	8
Loimia viridis	1	1	1	4	7
Scoloplos rubra	0	3	2	1	6
Mediomastus californiensis	2	2	1	0	5
Malacoceros vanderhosti	2	1	0	0	3
Aricidea fragilis	1	0	1	0	2
Armandia agilis	0	0	2	0	2
Axiothella mucosa	1	0	0	1	2
Nephtys picta	0	1	1	0	2
Prionspio heterobranchia	1	0	1	0	2
Unidentified Sabellidae	0	1	0	1	2
Amphictene sp.	1	0	0	0	1
Galathowenia sp.	0	0	1	0	1
Glycera americana	0	0	1	0	1
Lumbrineris sp.	0	1	0	0	1
Magelona rosea	0	0	0	1	1
Minuspio sp.	0	1	0	0	1
Neanthes succinea	0	1	0	0	1
Pectinaria gouldii	0	1	0	0	1
Total annelids	17	15	13	15	60
Rhynchocoela					
Unidentified nemertean	5	1	2	0	8
Cerebratulus lacteus	0	1	2	3	6
Total rhynchocoels	5	2	4	3	14

TABLE III

Continued.

Taxon	Control	DBP (mg/l)			Total animals
		0.036	0.45	3.8	
Arthropoda					
Tanaidacea (Apseudidae)	1	2	2	1	6
Unidentified Paguridae	3	0	0	0	3
Pinnixa cristata	0	1	0	1	2
Neopanope texana	0	1	0	0	1
Sicyonia laevigata	1	0	0	0	1
Total arthropods	5	4	2	2	13
Chordata					
Branchiostoma caribaeum	2	3	4	0	9
Myrophis punctatus	0	1	0	0	1
Total chordates	2	4	4	0	10
Echinodermata					
Ophiophragmus filograneus	0	2	2	0	4
Coelenterata					
Unidentified Actiniaria	0	0	1	0	1
All phyla					
Individuals	54	49	55	23	181
Species	25	28	27	13	46

TABLE IV

Average density of animals and average number of species (in parentheses) per aquarium collected from field-colonized control aquaria and from aquaria that received DBP for 14 days.

Phylum	Control	DBP (mg/l)		
		0.036	0.45	3.8
Mollusca	3.1	2.8	3.6	0.4*
	(2.4)	(2.1)	(2.9)	(0.4*)
Annelida	2.1	1.9	1.6	1.9
	(1.8)	(1.8)	(1.6)	(1.6)
Rhynchocoela	0.6	0.3	0.5	0.4
	(0.5)	(0.3)	(0.4)	(0.4)
Arthropoda	0.6	0.5	0.3	0.3
	(0.5)	(0.5)	(0.3)	(0.3)
Chordata	0.3	0.5	0.5	0
	(0.1)	(0.5)	(0.4)	(0)
Echinodermata	0	0.3	0.3	0
	(0)	(0.3)	(0.3)	(0)
Coelenterata	0	0	0.1	0
	(0)	(0)	(0.1)	(0)
All phyla	6.8	6.1	6.9	2.9*
	(5.3)	(5.4)	(5.9)	(2.6*)

* Significantly different from control at the 5% level.

DISCUSSION

Data from laboratory-colonized communities indicated that as little as 0.34 mg DBP/l can significantly affect amphipods and 3.7 mg/l can affect many taxa and cause impoverishment of assemblage composition. Although field-developed communities were dominated numerically by only two phyla, mollusks and annelids, they provided corroborating data on DBP effects. Average number of species per aquarium was reduced by the highest concentration in both laboratory- and field-developed communities. Also, in each situation, assemblages of mollusks were significantly smaller in the highest concentration than in the control or lower concentrations of DBP. Of 6 mollusk species colonizing in both laboratory and field, 5 did not occur at the highest DBP concentration in either experimental group. Chemical effect probably was species-dependent since annelid abundance in the highest concentration differed from the control only in laboratory-colonized communities. *Armandia agilis*, the dominant and most affected annelid in laboratory assemblages of animals, was represented by only two individuals in field assemblages.

Effects on deposit feeders observed in sand communities might be more pronounced in mud communities. DBP probably would adsorb onto the organic and greater surface area of mud particles to a greater degree than onto sand particles.

Since we used no solvent-free control, any effects of acetone in our dilution water (0.3 ml/l) on communities were not determined; the effects of acetone on bioavailability of DBP are also unknown. While it is possible that bioavailability and toxicity of DBP would be affected by acetone, we know of no data supporting this possibility. Concentration of acetone used was less than the maximum recommended (0.5 ml/l) by ASTM (1980) and less than that (5540 mg/l) toxic to trout (Johnson and Finley, 1980).

Field communities contained more species than laboratory communities, 46 compared to 29. Fourteen species were in common to the laboratory and field communities. Abundance of individuals and species agreed with those of earlier comparative studies of laboratory- and field-developed communities that showed that the former had more animals of fewer species than the latter (Tagatz and Deans, 1982).

Although the toxicity of DBP to aquatic organisms is less than many other contaminants, its high production and widespread use in plastics add to the potential of a serious environmental pollutant. Marx (1972) reported that phthalates have occurred in parts per billion (μg/l) concentrations in Atlantic and Gulf coast rivers and that specimens of fish, crustaceans, and water collected throughout North America were found to contain DBP. He also stated that experimental data implied that this phthalate can accumulate in the food chain. Results of acute tests on certain freshwater animals and our community studies on estuarine species indicate that very low ppm concentrations of DBP are toxic to a variety of taxa. It appears to

be even more toxic to saltwater algae. Wilson et al. (1978) calculated 96-h EC_{50} values as low as 3.4 μg/l (chlorophyll *a*) and 20 μg/l (cell number) for *Gymnodinium breve* exposed to DBP. Although further chronic testing is needed to determine minimal concentrations of DBP that are injurious to aquatic organisms, known toxic effects are sufficient to warrant concern over current and potential concentrations that enter our aquatic environments.

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